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Several species of commercially valuable marine macroalgae (Farlowia mollis, Gigartina exasperata, Gracilaria lemaneiformis, Iridaea cordata, Palmaria mollis, Porphyra abbottae, and Porphyra nereocystis) were screened to assess their growth in effluent water from pump-ashore salmon mariculture systems. Species screening and stocking density experiments were conducted in small tanks (0.13 $m^2$ surface area) receiving 40 volume changes $d^{-1}$ of effluent. Of the seven species tested, P. mollis was found to be best suited for culture in effluent from salmon culture based upon its observed specific growth rate, persistence in culture, resistance to epiphytes, ease of culture, and market potential. Maximum productivity of P. mollis (15.7 g dry weight $m^{-2}$ $d^{-1}$) occurred at a stocking density of about 10 g wet weight $l^{-1}$. P. mollis reduced ammonia concentration by 60% and phosphorus concentration by 32% at stocking densities of about 10 g wet weight $l^{-1}$. Light,

not available nutrients, was found to limit growth of *P. mollis* in tanks receiving 80 volume changes $d^{-1}$ at the stocking density which produced maximum yield.

# Land-based Polyculture of Marine Macroalgae and Pacific Salmon

by

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# IN MEMORIAM

Two of my colleagues, both of whom made significant contributions towards the completion of this thesis, passed away during the coarse of this study. They deserve a special place in this thesis to acknowledge their efforts. They were loved, and they are deeply missed.

Marilyn Guin generously served the Marine Science Center as its librarian. She often amazed me as she would lead me through the stacks of books and hand me the documents I was seeking before I had finished telling her what it was. She would then unfold a Chesire cat's smile and hand me another book that I didn't even know I needed, yet its value to me was greater than the text I had originally sought. Marilyn was keenly aware of what everyone at the Science Center was studying and showed genuine interest in their research. She was an exceptional person, professionally, and socially.

Dennis Lund was a co-conceiver of this project, yet his most important contributions to this research were his knowledge and wisdom that he passed to me. During the few months we worked side by side he would pass along bits of information such as how a pump should be repaired, when a raceway should be cleaned, or his ideas for future research. I became aware that Dennis, recognizing his mortality, was handing down lists of information that I

would need to keep our program running smoothly. It was through his attitude, however, that he taught me more than I could ever hope to learn from books or in a classroom. It is to his memory that this thesis is dedicated.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

# LAND-BASED POLYCULTURE OF MARINE MACROALGAE AND PACIFIC SALMON

## INTRODUCTION

Raising salmon in floating net pens has become increasingly popular among U.S. salmonid culturists. This practice, however, has exacerbated conflicts over limited space available in bays and estuaries for commercial fisheries, jurisdictional, wildlife, navigational, and public use (Butler, 1986; Mumford, 1987). Several counties in the state of Washington have instituted a moritorium on new salmon farming permits despite much commercial interest in expansion. Arguments favoring the moritorium are based largely on concerns over maintaining water quality and property values (Aquaculture Digest, 1987a,c). Alaska has also recently curbed salmon farming, largely because of the powerful commercial fishing lobby (Aquaculture Digest, 1987b). In Oregon, farming in floating cages would be limited due to the lack of suitable estuarine sites. These conflicts may lead to a shift from net pen culture to land-based raceway methods, which are currently being practiced in the Pacific Northwest. Most of Oregon's farmed salmon are currently grown in land-based raceways (Aquaculture Digest, 1986).

Effluent from land-based salmonid facilities may impact receiving waters. Farmed salmon are fed prepared

diets with a high protein content. Metabolic wastes of fish raised in this manner are high in inorganic nitrogen (N) and phosphorus (P). Addition of these nutrients to estuaries may alter algal community dominance (Waite and Mitchell, 1972), and produce more plant material than can be consumed by grazing animals (Harlin and Thorne-Miller, 1981). Concern has been expressed over possible eutrophication of bays and estuaries due to excess nutrients discharged from land-based sites and the potential for depletion of dissolved oxygen in receiving waters as excess plant material decomposes. Particulate and dissolved organic matter in fish farm effluents will also cause low dissolved oxygen concentrations. Additionally, effluent water of animal cultivation facilities has low dissolved oxygen concentrations as a result of respiration by cultured animals.

The ability of marine macroalgae to remove inorganic nutrients from their medium has been well documented in both field and laboratory experiments. Little effort, however, has been expended on evaluating the feasibility of utilizing seaweeds to remove nutrients in an aquacultural context. Prince (1974) and Ryther et al. (1975) employed marine macroalgae for removal of inorganic nutrients from secondarily treated sewage mixed with seawater. Harlin (1978) suggested using seaweed to remove $NO_3^-$ from aquaculture systems. Harlin et al. (1979) demonstrated the ability of seaweed to take up $NH_4^+$ produced by fish in a

recirculating aquarium. Despite encouraging results of these investigations, integration of fish and algal cultures on a commercial scale has not been undertaken. This project examined the feasibility of raising commercially valuable seaweed in effluent of land-based salmon operations. Algal species considered to have aquaculture potential are described in detail below and summarized in Table 1.

Farlowia mollis (Harvey & Bailey) Farlow & Setchell, Holden & Setchell 1901 has not been extensively studied because it currently has no commercial value. This species was tested, however, because of growing interest in its anti-microbial/anti-viral constituents (W. Gerwick, pers. comm.). F. mollis ranges from Macleod Harbor, Prince William Sound, Alaska, to San Martin I., Baja California, Mexico (DeCew and West, 1981). F. mollis used in this study was collected from Otter Crest, Oregon.

Porphyra nereocystis Anderson in Blankinship & Keeler 1892 occurs naturally as an epiphyte on the annual kelp Nereocystis luetkeana (Woessner, 1981). P. nereocystis has been cultured without its host (Dickson and Waaland, 1985). Temporal abundance of P. nereocystis and its host vary regionally (Woessner, 1981). The range of P. nereocystis is from Unalaska I., Aleutian Is, Alaska, to San Luis Obispo County, California (Scagel et al., 1986). P.

nereocystis used in this study was collected from Fogarty Creek State Park, Oregon.

P. nereocystis is considered to be an excellent candidate as an aquaculture food product (nori) because of its flavor, texture, and size (Dickson and Waaland, 1984). Thalli approach 4 m in length and 1 m in width (Hawkes, 1978). Most studies of P. nereocystis have been concerned with the conchocelis stage (Dickson and Waaland, 1984; 1985), therefore little is known about nutrient uptake and growth rates of thalli. Photosynthetic saturation would be expected to occur at a low irradiance level since in nature P. nereocystis is shaded by the fronds of N. leutkeana.

An additional Porphyra species, Porphyra abbottae Krishnamurthy 1972, was also tested. Studies of this species have been limited to the conchocelis phase (Waaland and Dickson, 1983; Waaland et al., 1984). This species could produce a more desirable food product than most other eastern Pacific Porphyra species (R. Waaland, T. Mumford, pers. comm.). It occurs from March to September (Gabrielson et al., 1987) in southeast Alaska, British Columbia, and northern Washington (Scagel et al., 1986). Fidalgo I., Washington was the source of P. abbottae used in this study.

Gigartina exasperata Harvey & Bailey 1851 is a temperate zone carrageenophyte. The distribution of G. exasperata is from northern Washington to Cabo San Quintin,

Baja California, Mexico (Scagel et al., 1986). Samples of G. exasperata used in this study were obtained from Fidalgo I., Washington.

Despite low growth rates of G. exasperata (3.35% fresh weight increase $d^{-1}$ in spring/summer, 2.22% fresh weight increase $d^{-1}$ in winter) (Waaland, 1979), it is considered a desirable culture species because the thalli do not degrade following sporulation (T. Mumford, pers. comm.). Waaland (1978) has demonstrated that strain selection for fast growing plants can yield sustained growth rates exceeding 4% fresh weight increase $d^{-1}$. Field tests have shown the feasibility of culturing this species in the Pacific Northwest (Waaland, 1973).

The red algae Palmaria mollis (Setchell & Gardner) van der Meer & Bird 1985, previously known as Palmaria (=Rhodymenia) palmata forma mollis, is found during summer to late fall from the Aleutian Is., Alaska, to San Luis Obispo County, California (Scagel et al., 1986). Fidalgo I., Washington was the source of P. mollis used in this study.

P. mollis may have value as a food crop (dulse) as does P. palmata (Davis, 1980b). P. palmata is a highly regarded food source, containing up to 25% protein on a dry weight basis and appreciable quantities of many minerals, notably iron and potassium, and vitamins B6 and B12 (Morgan and Simpson, 1981a; Loomis, 1989).

Laboratory tests have demonstrated that P. palmata grows equally well (6 - 8% fresh weight increase $d^{-1}$) at temperatures between 6 - 14 $^{o}$C (Morgan and Simpson 1981a; 1981b) with an optimum for photosynthesis at 10 - 15 $^{o}$C. Photosynthesis is saturated at approximately 250 $\mu$E $m^{-2}$ $s^{-1}$ (Robbins, 1979). Maximum photosynthetic rates were achieved at a pH of 6.5 - 7.5 and a salinity of 32 $^{o}$/oo (Robbins, 1979).

The high carrageenan content (52 - 66% dry weight) and rapid growth rate (9.5% fresh weight $d^{-1}$) of Iridaea cordata (Turner) Bory 1826 (Waaland, 1978; Mumford, 1979) make this species an attractive candidate for mariculture. Growth experiments in the field (Mumford, 1979) and in tanks (Waaland, 1978) have demonstrated that culture of I. cordata is feasible. A drawback is its rapid degeneration following sporulation in late summer (T. Mumford, pers. comm.). The natural distribution of I. cordata is from the Aleutian Is., Alaska to Baja California, Mexico (Scagel et al., 1986). I. cordata used in this study was collected at Boiler Bay, Oregon.

Gracilaria lemaneiformis (Bory) Weber-van Bosse 1928, previously known as G. sjoestedtii Kylin, may be the seaweed best suited for polyculture with salmonids (T. Mumford, pers. comm.). This species is very tolerant of salinity and temperature fluctuations due to its intertidal existence. Other species in this genus are regarded as

nutrient sponges, capable of removing large amounts of $NH_4^+$ from their media (Nelson et al., 1980).

Gracilaria species typically have rapid growth rates due in part to their high rates of nutrient uptake. Nelson et al. (1980) reported that G. edulis and G. arcuata grown in shallow tanks in Guam had a SGR (specific growth rate = % fresh weight increase $d^{-1}$) of up to 7.6. Ammonia uptake was linearly related to substrate concentration up to 5.0 x $10^3$ µg-at $NH_4^+$-N $l^{-1}$. Levels of $NH_4^+$ exceeding 60 µg-at $NH_4^+$-N $l^{-1}$ are commonly toxic to marine macroalgae. The SGR of G. lemaneiformis reared in tank culture in Elkhorn Slough, California was 8 - 9 (Hanson, 1983). G. secundata grown in cylindrical tanks receiving constant illumination from submerged light sources obtained a SGR of 47 (Lignell et al., 1987). In the Pacific Northwest, growth of seaweeds is often light limited (J. Waaland, pers. comm.); however, Hansen (1984), demonstrated that a decrease in irradiance resulted in a higher SGR for G. lemaneiformis cultured in Monterey Bay, California, presumably due to a reduction of epiphytes on plants grown in lower light treatments. Plants receiving 33% of mid-day surface irradiance had a 10% higher SGR than those grown at 84% mid-day surface irradiance. However, agar content of algae grown at low light levels was only half that of those grown at higher light intensities. Net photosynthesis was saturated between 475 and 500 µE $m^{-2}$ $s^{-1}$.

Gracilaria sp. are primarily cultured for agar, though they are also used as fodder (Chen, 1984) and human food (R. Fujita, pers. comm.). The 49.91% yield (weight:weight, anhydrous weight) of agar from G. lemaneiformis compares favorably to the 38.1 to 42.0% yields obtained from G. verrucosa (Durairatnam and de Queiroz Santos, 1981). Craigie et al. (1984) found G. lemaneiformis agar to be superior to that of G. tikvahiae, G. textorii, and G. verrucosa based upon gel clarity, strength, and chemical constituents. Whyte and Englar (1979) found that agar content of Gracilaria sp. cultured in tanks receiving either unenriched seawater or effluent from a tank holding halibut was higher than that of naturally occurring algae. No significant difference in agar content existed between the two cultured stocks. Productivity of algae receiving halibut effluent, however, was more than twice that of seaweed grown in seawater. Additionally, high N levels typical of salmon culture effluent tend to increase agar gel strength (Bird, 1988).

According to Guerin and Bird (1987), Gracilaria sp. do not require constant aeration in order to grow well in concrete tanks. They reported that productivity of algae in tanks receiving 12 h of aeration per day did not significantly differ from those with constant aeration. Growth of algae in tanks receiving six or less hours of aeration per day was significantly lower than tanks

receiving aeration of 12 and 24 hours per day. Aeration schedule did not affect gel content, strength, gelling or melting temperature. A major operating expense of an aquaculture system raising Gracilaria could therefore be greatly reduced.

G. lemaneiformis ranges from Vancouver I., British Columbia to Mexico (Scagel et al., 1986). Hansen (1984) reports the production of natural populations of G. lemaneiformis to be highest from late fall and winter. This may simply reflect the inability of this species to compete with opportunistic Ulva and Enteromorpha species during spring and summer (M. Davis, pers. comm.). It is believed that this algae will achieve high growth rates in spring and summer when maintained in unialgal culture (R. Fujita, pers. comm.). G. lemaneiformis used in this study was collected from Yaquina Bay, Oregon.

Table 1. Summary of marine macroalgae tested in this research.

| Species | Type | Use | Seasonal Availability |
|---|---|---|---|
| *Farlowia mollis* | red | biomedical | unknown |
| *Porphyra nereocystis* | red | food | fall-spring |
| *Porphyra abbottae* | red | food | spring-fall |
| *Gigartina exasperata* | red | carrageenan | perennial |
| *Palmaria mollis* | red | food, fodder | summer-fall |
| *Iridaea cordata* | red | carrageenan | spring-summer |
| *Gracilaria lemaneiformis* | red | agar | fall-winter |

## MATERIALS & METHODS

### Culture apparatus

In all experiments, algae were reared in 11.5 l polyethylene test cells (surface area = 0.13 $m^2$) receiving effluent from a 12,900 l circular tank containing coho salmon stocked at a density of 9 kg $m^{-3}$. Fish ration and water exchange rates were maintained at levels typical of a commercial operation. Salmon were fed a commercial ration (Silver Cup, Murray Elevators, Murray, Utah) at a rate of 2.5% of body weight per day during experiments in spring summer and fall, but daily feeding rate was decreased to 1.75% of body weight in winter. Food was delivered to fish by an automatic feeder over a 12 h period each day. When less than 12 h of light was available, feeding was programmed so that all food was presented to fish before darkness. Salmon effluent flowed into a settling tank and was then pumped into a 400 l light-protected reservoir and delivered by gravity to a PVC pipe grid suspended over the algal test cells. Flow of sea water enriched with fish waste into each test cell was controlled by an adjustable nozzle attached to the PVC pipe. A perforated polyethylene bottle was placed over the effluent pipe of each test cell to prevent algae from escaping.

Seaweed was maintained in suspension by bubbling compressed air from perforated tubing secured to the bottom of the test cells. Aeration provided a number of benefits, including increased nutrient uptake by disruption of

boundary layers at the algal surface - water interface (D'Elia and DeBoer, 1978), increased exposure of algae to light (Guerin and Bird, 1987), reduced sediment and epiphyte settlement on seaweeds (DeBusk et al., 1986) and oxygenation of the water (J. Levin, unpublished). Dye studies indicated that aeration efficiently mixed the water as it flowed through each test cell.

Algal growth determinations

Algal biomass was determined by removing algae from test cells, rinsing with filtered seawater to remove epiphytes, centrifuging to remove excess water, and weighing to the nearest 0.01 g. After weighing, algae were returned to the test cells at the initial stocking density. Excess material was rinsed in fresh water, spun, weighed, and dried at 60 $^{o}$C to a constant weight to establish dry weight:fresh weight ratios.

Growth was expressed in terms of specific growth rate (SGR = % fresh weight increase $d^{-1}$) and productivity (g dry weight $m^{-2}$ $d^{-1}$). SGR was calculated using the equation

$$SGR = 100\ [\ln\ (w_2/w_1)]/(t_2-t_1)$$

where $w_1$ and $w_2$ were wet weights (g) at times $t_1$ and $t_2$ (d), respectively.

For each experiment, macroalgae were grown in test cells under experimental conditions for two weeks prior to collection of growth and nutrient uptake data. During this time seaweeds exhausted their stored nutrient reserves

(Morgan and Simpson, 1981b) so experimental results reflected effects of treatment conditions.

Nutrient uptake analyses

To determine the concentration of N and P in seawater, samples were collected in 125 ml polyethylene bottles (Wheeler, 1985) that had been rinsed three times with sample water before filling. Immediately after collection, the water was filtered through a Whatman GF/F filter. A vacuum of less than 150 mm Hg was employed to minimize damage to cells and consequent release of intracellular nutrients (Wheeler, 1985).

Nitrate + nitrite, ammonia, and phosphorus concentrations of each sample were determined according to spectrophotometric methods outlined in Wheeler (1985). Two aliquots were measured for each sample and standard. Descriptions of reagent solutions are presented in the appendix.

To determine $NO_3^-$ + $NO_2^-$ concentrations a portion of the sample was passed through a cadmium-copper reduction column and collected in a beaker. Ten ml of sample was added to a reaction tube. Addition of 0.2 ml NEDA solution and 0.2 ml sulfanilamide solution to each reaction tube produced a red color. The color intensity is dependent upon the amount of $NO_2^-$ formed from the reduction of $NO_3^-$ originally present in the sample plus background $NO_2^-$ concentrations. Background $NO_2^-$ concentrations were not

determined as this nutrient is typically present in low quantities and not considered a major form of inorganic nitrogen in seawater (Wheeler, 1985). Absorbance was measured using a spectrophotometer at 543 nm within two hours of addition of reagents. Samples could have been stored in a refrigerator for up to 24 hours with no significant loss of color intensity (J. Jamula, pers. comm.). Nitrate + nitrite concentrations were calculated by comparing sample absorbances with a standard curve which covered the range of $NO_3^-$ + $NO_2^-$ values examined. Five known concentrations of reagent grade $KNO_3$ were used to generate a standard curve.

Ammonia concentrations were determined according to the phenolhypochlorite method of Solorzano (1969). Addition of 0.4 ml phenol solution, 0.4 ml nitroprusside solution, and 1.0 ml oxidizing solution to 10 ml of each sample produced a blue color. The solution was mixed well following the addition of each reagent and stored in the dark for 1 to 24 hours for color development. Absorbances were measured at 640 nm and $NH_4^+$ concentrations calculated as described above. Reagent grade $NH_4Cl$ was used to prepare standard solutions.

Phosphorus concentrations were determined by adding 1.0 ml of mixed reagent (2 parts ammonium molybdate solution, 5 parts sulfuric acid solution, 2 parts citric acid solution, and 1 part potassium antimonyl tartrate

solution) to 10 ml of each sample. Absorbances were measured at 885 nm 5 to 30 minutes after addition of reagents. Comparison of sample values with those of standards prepared from reagent grade $KH_2PO_4$ allowed determination of $PO_4^{3-}$ concentrations.

Absorbance of each sample was measured in triplicate. The spectrophotometer was recalibrated with a distilled deionized water (DDW) blank after measuring each sample.

Species screening experiments

Initially, different species of seaweed were tested to determine their suitability for culture in salmon effluent. This assessment was based upon their SGR, nutrient removal ability, persistence in culture, resistance to epiphytes, ease of culture, and potential use to aquaculturists as either a saleable harvest or fodder for grazing marine herbivores such as abalone and urchins.

Water flow into algal test cells was maintained at 0.32 l $min^{-1}$ (40 test cell volume changes per day). High flow rates such as this have been reported to maximize algal growth rates (DeBusk and Ryther, 1984; Harlin and Wheeler, 1985).

Algal species were initially added to test cells at the low density of 1 g $l^{-1}$ (0.09 kg $m^{-2}$) to maximize growth (Fujita, pers. comm). Algae were weighed twice weekly as described above. The contents of each test cell were centrifuged for 15 seconds in a salad spinner. This method

of centrifugation of algae in batches up to 35 g produced highly repeatable weights (std. dev. < 3%). Three replicate test cells were set up per species.

Water samples were collected for nutrient analyses prior to removing algae from test cells for weighing. Nutrient analyses were conducted for samples collected at the inflow to the tank holding salmon, from a stand pipe in the PVC grid delivering seawater to algal test cells, and from the effluent tube of each test cell. All water samples were filtered and subsamples were immediately used to determine $NH_4^+$ concentrations (Wheeler, pers. comm.). The remainder of each sample was stored in a polyethylene bottle at 4 $^{o}$C for later analyses. Effluent phosphorus concentrations were analyzed after algae had been weighed. Nitrate + nitrite uptake was not measured during species screening experiments. Previous work had shown that concentrations of nitrate + nitrite in salmon culture effluent was not higher than that of incoming seawater and, therefore, these nutrients are not significant pollutants of salmon farms (J. Levin, unpublished).

Water temperature, pH, salinity, and irradiance were measured for each tank twice weekly. Irradiance was measured at the water surface of the test cells using a LI-COR quantum/radiometer/photometer (model # LI-185B). Measurements were taken at dawn, mid-day, and dusk on each sampling date.

Several species tested were summer annuals and not available for year-round testing. Therefore, both spring/summer (24 June 1988 - 1 september 1988) and fall/winter (29 November 1988 - 10 January 1989) screening experiments were conducted using available species.

Ambient irradiance levels were reduced 84% by covering test cells with mesh during spring/summer experiments. This was necessary to minimize water temperature rise within test cells and to decrease epiphytic growth on test species. No covers were used during fall/winter experiments.

Stocking density experiment

The macroalgal species determined by screening experiments to be best suited to culture in salmon effluent was used in an experiment designed to determine the stocking density at which algal productivity was maximized. Six algal stocking densities (1.13, 5.65, 10.17, 14.70, 19.22, and 23.74 g wet weight $l^{-1}$) and a constant effluent flow rate (40 test cell volume changes per day) were used in the stocking density experiment. Algal stocking densities are typically reported as kg wet weight $m^{-2}$. The highest density (23.74 g $l^{-1}$) was one in which algae filled almost the entire volume of the test cell. The corresponding stocking density expressed in terms of per unit surface area equals 2.10 kg wet weight $m^{-2}$, which is a relatively low stocking density compared to literature

values. The low algal biomass to test cell surface area ratio was due to the high surface area to volume ratio of test cells. All treatments had three replicates. The experiment was conducted from 24 March 1989 to 10 May 1989. Ambient light to test cells was reduced by 84% by covering cells with screen as described in "species screening experiments".

Following the standard acclimation period of 14 days, algae were weighed once or twice per week for four weeks. A salad spinner was not large enough to centrifuge algae from high stocking density treatments; therefore, algae from all treatments were placed in nylon mesh bags and centrifuged in an automatic washing machine set on the spin cycle. The contents of several test cells could be centrifuged at one time since they were in separate mesh bags. Spinning algae for three minutes resulted in highly repeatable weights (std. dev. < 1%).

Ammonia and phosphorus analyses were conducted as described above.

Limiting factor experiment

A factorial experiment (2 nutrient loading levels x 4 light levels with 3 replicates per treatment) was conducted to determine whether nutrients and/or irradiance limited growth at the stocking density determined to optimize productivity in the previous experiment.

Flow rates of salmon effluent were adjusted to either 80 or 40 test cell volume changes per day. To eliminate confounding effects of flow rate, nutrient-free sea water was added to test cells receiving 40 volume changes per day of salmon effluent so that all test cells received 80 volume changes per day. This nutrient-free water was obtained by passing sea water through several cultures of Ulva before it was pumped to a head tank and delivered to test cells through a PVC pipe grid as described in "culture apparatus".

Irradiance levels were varied by shading test cells with different screens. The four irradiance levels used were 100%, 68%, 47%, and 16% of ambient. Several times per week ambient irradiance was measured using a LI-COR LI-1800 Portable Spectro-radiometer (Behrenfeld, 1990).

Seaweed biomass was measured each week for four weeks (11 October - 9 November, 1989) following the 14 day acclimation period. Methods for measuring biomass and growth are described in "algal growth determinations".

At each weighing, algal samples were taken from individual test cells to determine total C, N, and P concentration of algal tissue. The tissue was dipped in a 0.5 M ammonium formate solution to remove external salts, freeze dried at -80 $^{o}$C, and further dried at 60 $^{o}$C for 48 h. This dried material was then ground to a powder in a Braun Mikro Dismembrator II. CHN analyses were carried out

using an organic elemental analyzer (Control Equipment Corporation model 240XA). This instrument combusts samples in an oxygen-enriched helium atmosphere, and analyzes combustion products ($CO_2$, $NO_x$, (reduced to $N_2$), and $H_2O$) with three pairs of thermal conductivity detectors. The machine is rated to provide a precision of +/- 0.3 weight percent. Each reported value results from one weighing/ combusting. These analyses were conducted by a technician from the University of California, Santa Barbara, Marine Science Institute analytical lab. Tissue P values were determined by a modified version of the alkaline persulphate digestion technique described by D'Elia et al. (1977). Dried algal tissue samples (500-700 ug) were put into silanized 50 ml screw cap test tubes. Five ml of DDW and 30 ml of oxidizing reagent (3.0 g NaOH and 6.7 g low N potassium persulphate dissolved in 1 l of DDW) were added to each tube. Samples were autoclaved (100-110 $^{o}$C at 15 psi) for 1 h. After cooling to room temperature, 3.0 ml of acid (0.3 M HCl) and 4.0 ml of buffer (30.9 g $H_3BO_3$ and 100 ml 1 M NaOH in 1 l DDW) were added to each tube. Eight ml of DDW were added to each tube to bring the volume to 50 ml. Phosphorus was then measured as described in "nutrient uptake analyses". Efficiency of digestion was determined by use of an organic standard, carbamyl phosphate (disodium salt).

Once per week water samples were drawn from the PVC grid every 2.5 - 4 h over a 24 hour period. Samples were frozen at -20 $^{o}$C and analyzed at a later date.

Statistical analyses

Prior to analyses, all data were tested for normal distribution by employing a normal probability plot. All data were found to be normally distributed.

Statistical methods used to evaluate results of each experiment varied according to the experiment. Non-parametric analysis was required for the summer screening experiment due to a loss of treatments and replicates. Therefore, Kruskal-Wallis multiple range test was employed to determine statistical significance of treatments. If parametric analyses could be applied, Cochran's C test was used to determine if variances of test groups were homogeneous. In cases where this was found not to be so, data were log transformed to achieve homogeneity. If a multifactor ANOVA test was required and there was an interactive effect of main effects, a Student-Newman-Keuls multiple range test was carried out for individual main effects. Kruskal-Wallis, ANOVA, and Student-Newman-Keuls tests were conducted at the 95% level. The effect of ambient irradiance intensity on SGR was determined by correlation analysis for the Limiting Factor experiment.

# RESULTS

## Species screening experiments

SGR, productivity, and nutrient removal rates of species examined during the summer screening trial are presented in Table 2. Some treatment replicates were terminated during the experiment due to excessive growth of epiphytes, namely chain-forming diatoms. *P. abbottae* and *P. nereocystis* were discarded before the acclimation period had been completed because of heavy fouling by epiphytes. It was only possible to collect water samples for nutrient analyses on two occasions, both near the conclusion of the screening experiment.

Cochran's C test revealed heteroscedasticity of variance for SGR ($P = 1.47 \times 10^{-14}$) and productivity ($P = 9.44 \times 10^{-15}$) which could not be remedied by log transformation. Heterogeneity of variances (when $P < 0.05$) and unequal replication dictated the use of a nonparametric test to evaluate results. Statistical significance of differences among SGRs and productivities were determined using Student-Newman-Keuls multiple range tests (95% confidence level) on the average rank values generated by a Kruskal-Wallis one-way analysis by ranks (Tables 3 and 4).

High standard deviations of SGR and productivities for *G. lemaneiformis* and *P. mollis* were due to a continuous improvement in growth response during the course of the experiment (i.e. high variation between sampling dates). High standard deviations of other species, however, were

Table 2. Summer species screening experiment (24 June - 1 September 1988): Average observed specific growth rate (% $d^{-1}$), productivity (g dry weight $m^{-2}$ $d^{-1}$), and nutrient removal by macroalgal species. n = number of algal growth measurements. Numbers in parentheses are 1 standard deviation. Mean influent [$NH_4^+$] = 21.28 µM (1 S.D. = 1.46, range = 20.24 - 22.31 µM). Mean influent [$PO_4^{3-}$] = 3.16 µM (1 S.D. = 1.59, range = 2.03 - 4.28 µM).

| Species | n | specific growth rate | productivity | mean % uptake of influent $NH_4^+$ | $PO_4^{3-}$ |
|---|---|---|---|---|---|
| F. mollis | 45 | 4.34 (2.28) | 0.64 (0.34) | 30.98 (1.14) | 11.72 (0.98) |
| G. exasperata | 46 | 2.44 (1.42) | 0.38 (0.27) | 29.30 (1.24) | 10.85 (0.99) |
| G. lemaneiformis | 48 | 6.86 (2.70) | 1.13 (0.48) | 31.83 (1.25) | 12.74 (1.26) |
| I. cordata | 45 | 2.40 (1.21) | 0.35 (0.20) | 28.91 (0.86) | 10.43 (0.74) |
| P. mollis | 48 | 10.26 (5.06) | 1.66 (0.90) | 33.17 (1.79) | 10.17 (1.11) |

Table 3. Summer species screening experiment (24 June - 1 September 1988): Kruskal-Wallis analysis of average rank values of macroalgal specific growth rates (% $d^{-1}$).

| Species | Average Rank | Homogenous Groups (95% level) |
|---|---|---|
| I. cordata | 60.778 | * |
| G. exasperata | 62.663 | * |
| F. mollis | 113.300 | * |
| G. lemaneiformis | 159.333 | * |
| P. mollis | 180.500 | * |

Table 4. Summer species screening experiment (24 June - 1 September 1988): Kruskal-Wallis analysis of average rank values of macroalgal productivities (g dry weight $m^{-2}$ $d^{-1}$).

| Species | Average Rank | Homogenous Groups (95% level) |
|---|---|---|
| I. cordata | 60.756 | * |
| G. exasperata | 66.185 | * |
| F. mollis | 109.733 | * |
| G. lemaneiformis | 161.906 | * |
| P. mollis | 177.917 | * |

caused by inconsistent growth and high SGR variation among sampling dates due to epiphytes (Figure 1).

Only G. exasperata, P. mollis, and G. lemaneiformis were tested during winter due to seasonal availability of species evaluated during the summer screening. During the middle of the acclimation period both G. exasperata and G. lemaneiformis were severely affected by epiphytic growth of diatoms. Various attempts were made to either remove diatoms or determine the amount of biomass attributable to host and epiphyte. As epiphytization progressed these efforts became impractical. The winter screening was therefore terminated on day 17 of the experiment because growth data could not be determined for these species. During this period P. mollis remained relatively free of epiphyte and grew rapidly (SGR = 9.17, S.D. = 1.23). Nutrient removal by each species is presented in Table 5.

Based on the results of screening experiments, it was determined that P. mollis was best suited for rearing in effluent of salmonid culture. This judgment was based on the consistently high SGR of P. mollis, its relative resistance to epiphytes, persistence in culture throughout the year, and ease of culture. This species was therefore used in an experiment to determine at what stocking density algal yield was maximized.

Stocking density experiment

Average observed algal SGR and productivity at each

Table 5. Winter species screening experiment (29 November 1988 - 10 January 1989): Nutrient uptake (% of test cell influent concentration removed) by each species. Numbers in parentheses are 1 standard deviation. Mean influent [$NH_4^+$] = 19.65 µM (1 S.D. = 0.15, range = 19.54 - 19.75 µM). Mean influent [$PO_4^{3-}$] = 3.12 µM (1 S.D. = 1.38, range = 2.14 - 4.09 µM).

| Species | $NH_4^+$ | $PO_4^{3-}$ |
|---|---|---|
| G. exasperata | 9.10 (1.23) | 8.10 (0.63) |
| G. lemaneiformis | 13.93 (2.99) | 9.81 (1.52) |
| P. mollis | 14.53 (2.76) | 8.28 (0.59) |

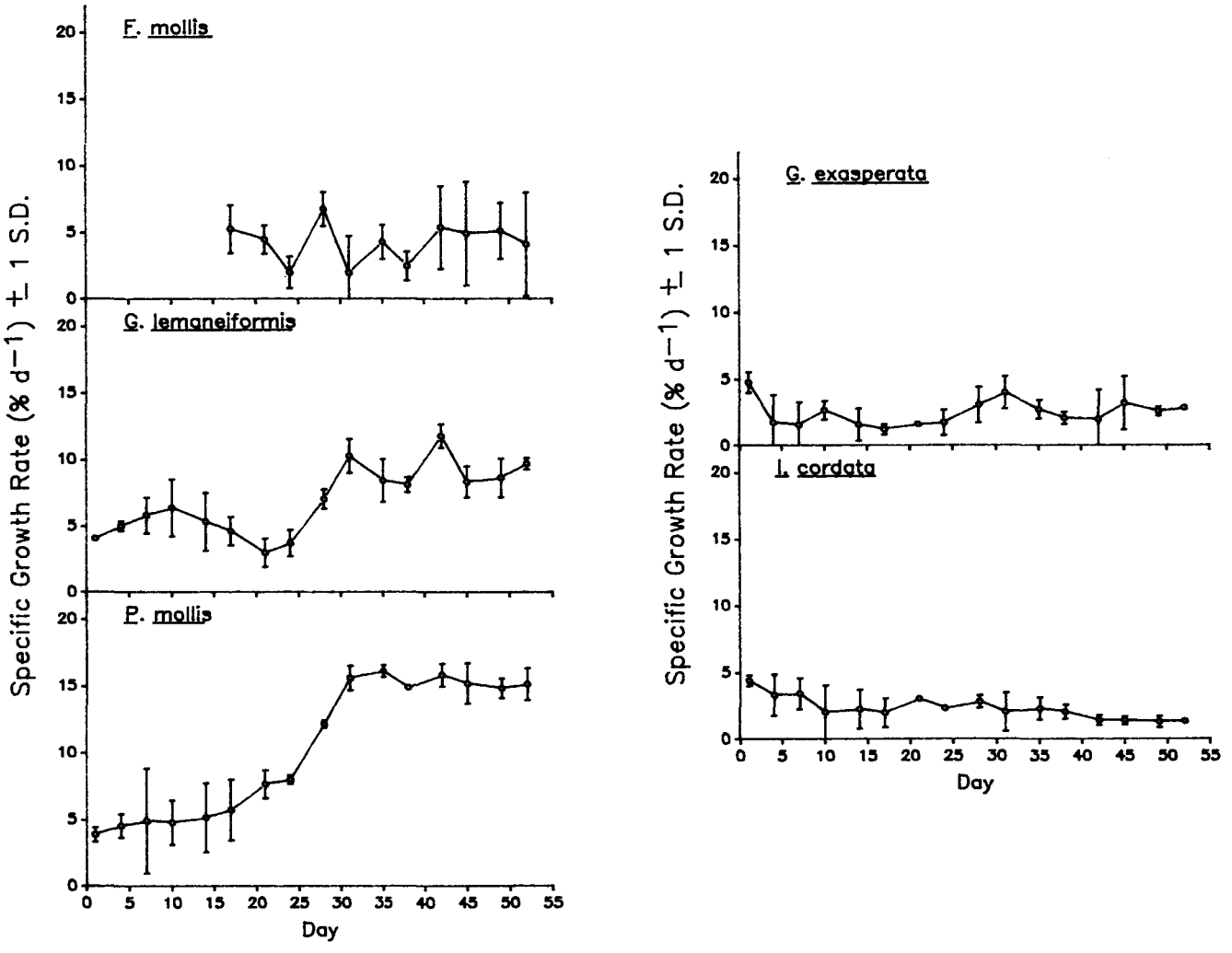


Figure 1. Growth rates of algal species during summer screening experiment. Sampling period: 12 July – 1 September 1988.

stocking density are presented in Figure 2. Treatment #1 (1.13 g $l^{-1}$) was terminated prior to the final sampling date due to heavy fouling by epiphytic diatoms. Therefore, mean SGR and productivity values reported for the stocking density of 1.13 g $l^{-1}$ are from the first five sampling dates. SGR and productivity of P. mollis on the sixth (final) sampling date were analyzed separately because of unequal treatment size.

An ANOVA of the mean SGR of plants at each stocking density revealed a highly significant difference in SGR as stocking density varied (dates 1 - 5, means transformed: $F = 295.85$, $df = 5$, $p < 0.001$; date 6: $F = 114.67$, $df = 4$, $p < 0.001$). Stocking density also had a highly significant effect on productivity. Mean productivities of algae at stocking densities 5.65, 10.17, and 14.70 g $l^{-1}$ were statistically identical ($\alpha = 0.05$) and significantly greater than those of other treatments (ANOVA, $F = 29.94$, $df = 5$, $p < 0.0001$).

Uptake of $NH_4^+$ and $PO_4^{3-}$ by P. mollis is presented in Figure 3. Student-Newman-Keuls multiple range tests on log transformed means of $NH_4^+$ and $PO_4^{3-}$ uptake values for individual sampling dates revealed that uptake values for algae at stocking densities 5.65 - 23.74 g $l^{-1}$ were generally not significantly different ($p < 0.05$), but all were significantly greater than algal uptake at 1.13 g $l^{-1}$ stocking density. The failure of macroalgae to remove greater amounts of nutrients at higher stocking densities

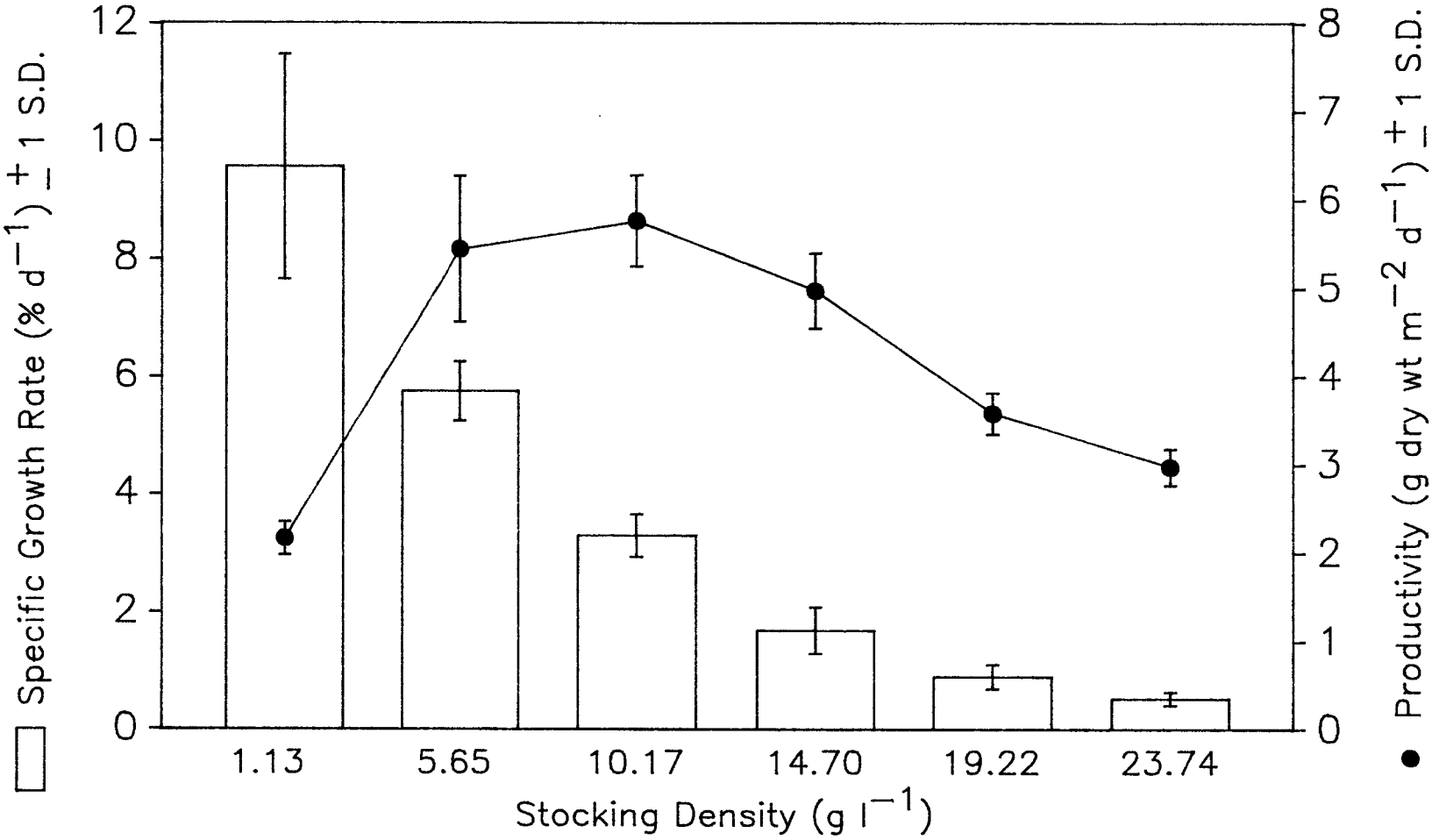


Figure 2. Growth rate ( ▭ ) and productivity ( ● ) of P. mollis vs stocking density. Sampling period: 12 April – 10 May 1989 (sampling period for 1.13 gl$^{-1}$ 12 April – 3 May).

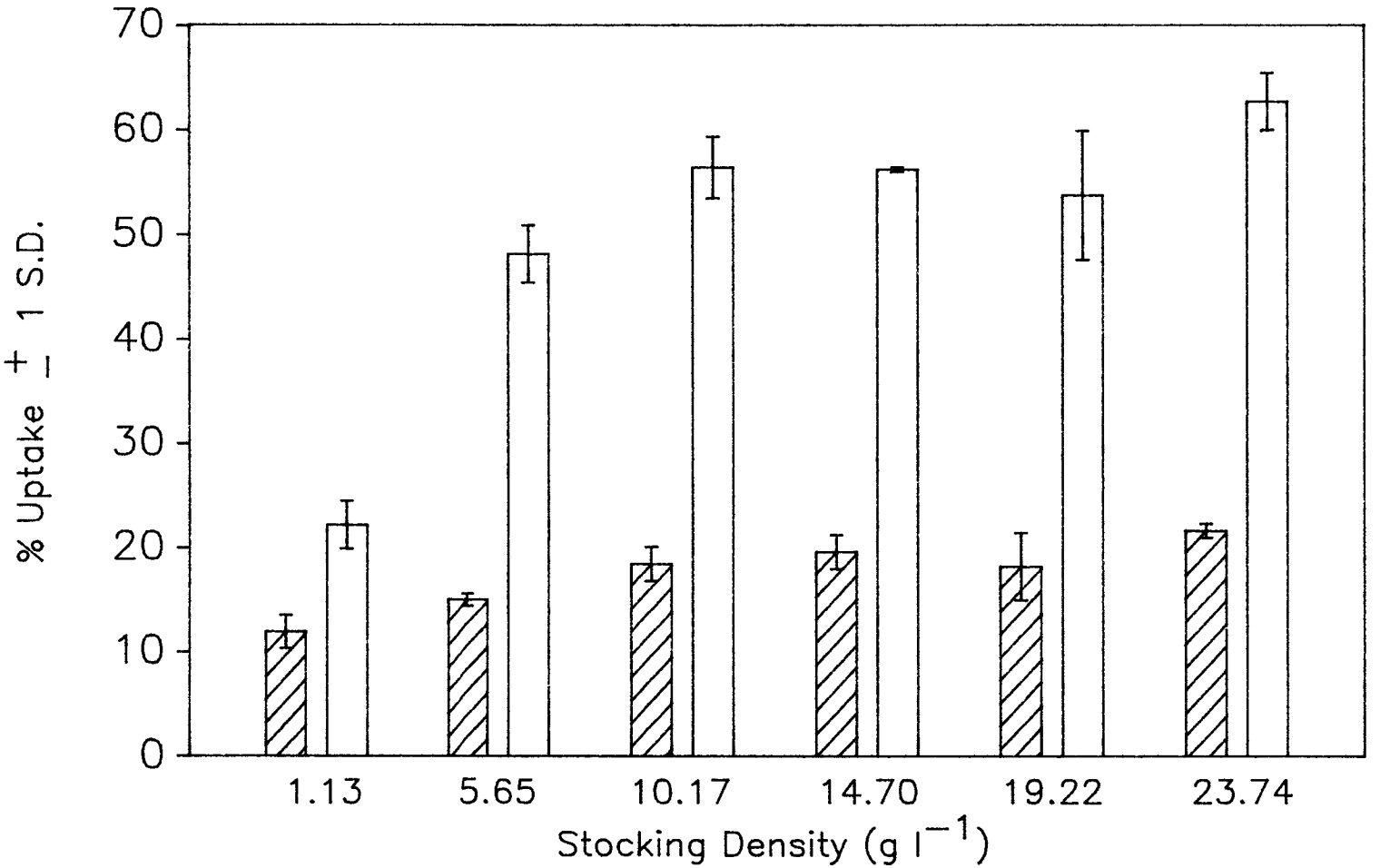


Figure 3. Uptake of $PO_4^{3-}$ ( ▨ ) and $NH_4^+$ ( ▭ ) by P. mollis during stocking density experiment. Mean influent $PO_4^{3-}$ concentration = 2.69 $\mu$M; $NH_4^+$ = 18.44 $\mu$M. Sampling period: 12 April – 10 May 1989 (sampling period for 1.13 $gl^{-1}$ 12 April – 3 May).

indicated some factor besides nutrient availability may be limiting uptake of nutrients by P. mollis.

Limiting factor experiment

P. mollis was cultured at an initial stocking density of 10.17 g $l^{-1}$ in the Limiting Factor experiment. Although this stocking density did not differ significantly in productivity from that of algae at 5.65 and 14.70 g $l^{-1}$ it was likely that this treatment would be less susceptible to epiphytes than lower stocked treatments because of increased self-shading. Furthermore, algae at a stocking density of 10.17 g $l^{-1}$ would likely have greater productivity at higher light levels than that of more densely stocked treatments due to better tumbling within the test cell.

SGR of P. mollis at each irradiance and flow level is presented in Figure 4. Irradiance level had a significant positive effect on macroalgal SGR. The impact of irradiance on SGR was analyzed using a Student-Newman-Keuls multiple range test ($p < 0.05$) on treatment means for each sampling date because of an interactive effect of sampling date. For sample dates 1, 3, and 4, there was a statistically significant different increase in SGR with an increase in irradiance level for all treatments. On sample date 2, SGR of algae at the 16% ambient light treatment was significantly less than that of algae from the 47% and 68% ambient light treatments, which were significantly less than that of algae from the 100% ambient light treatment.

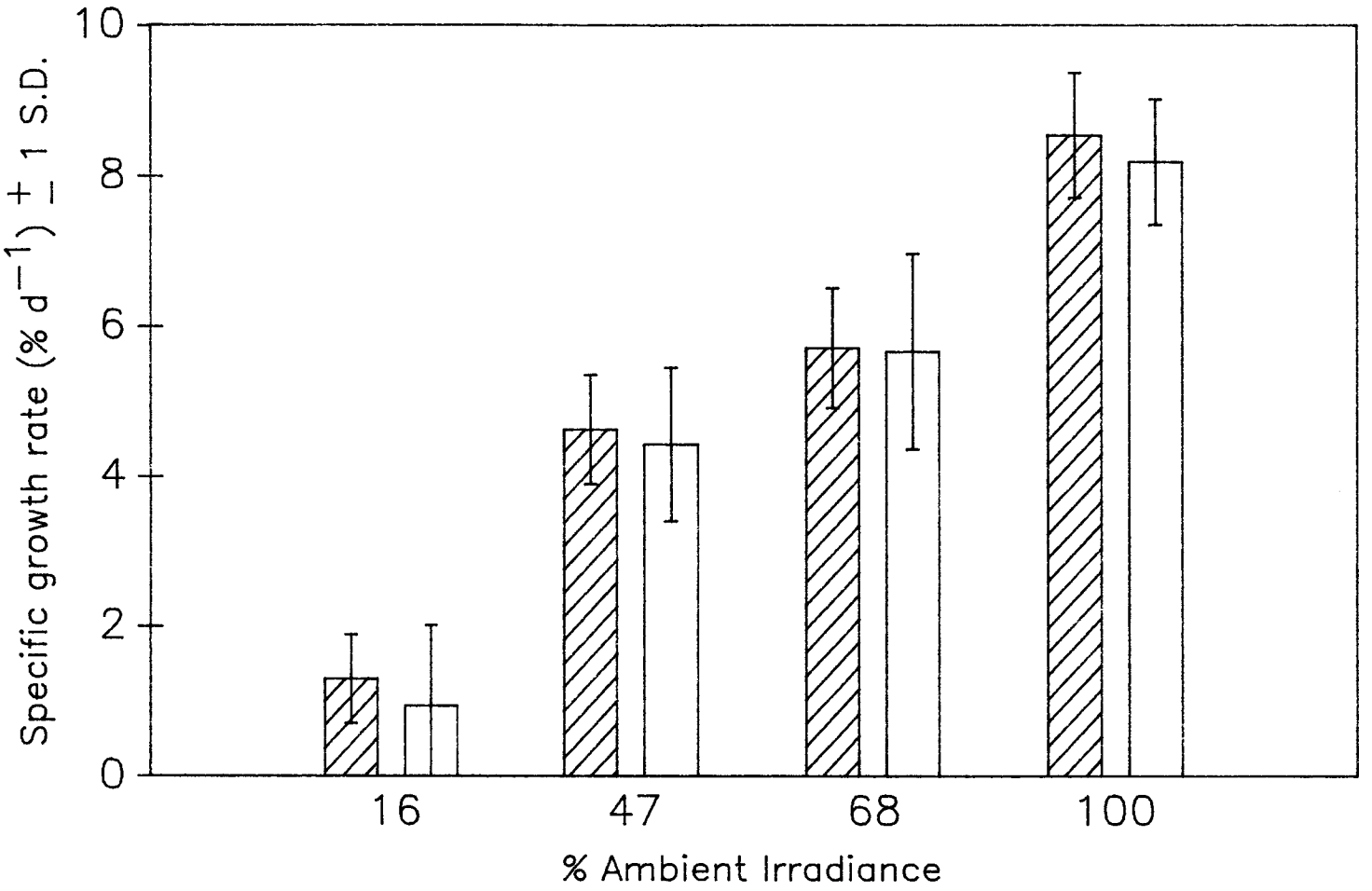


Figure 4. Effect of irradiance and nutrient regime on growth rate of P. mollis. Nutrient regime = 40 ( ▨ ) or 80 ( □ ) test cell changes d$^{-1}$ of salmon effluent. Sampling period: 11 October – 9 November 1989.

Within a light treatment, however, an increase in nutrient input had a slight negative effect on SGR (ANOVA, $F = 4.59$, $df = 1$, $p < 0.05$), possibly due to an increase in epiphytic growth in the richer nutrient media. Productivities of algae from each light treatment were likewise significantly different and therefore not presented. A maximum productivity of 15.7 g dry weight $m^{-2}$ $d^{-1}$ was obtained from algae in the ambient light - reduced nutrient treatment.

There was a significant interaction between sampling date and SGR for all treatments. This is explained by differences in average daily irradiance levels during each sampling period. There was a significant positive correlation between SGR and irradiance level ($r = 0.951$, $df = 2,2$; $p < 0.05$).

Phosphorus, ammonia, and nitrate + nitrite concentrations for both salmon effluent and seawater stripped of nutrients are presented in Figures 5, 6, and 7, respectively, for each sampling date. Although water flow rates and fish ration levels were strictly controlled, concentrations of nutrients in salmon effluent varied greatly between sampling dates, a problem which is inherent to open systems. An estimate of nutrient flux could not be made because concentrations of dissolved inorganic N and P in salmon effluent varied unpredictably with time.

An alternative approach to evaluating nutrient availability for algal growth is to determine nutrient concentrations in algal tissues and ratios of nutrients to

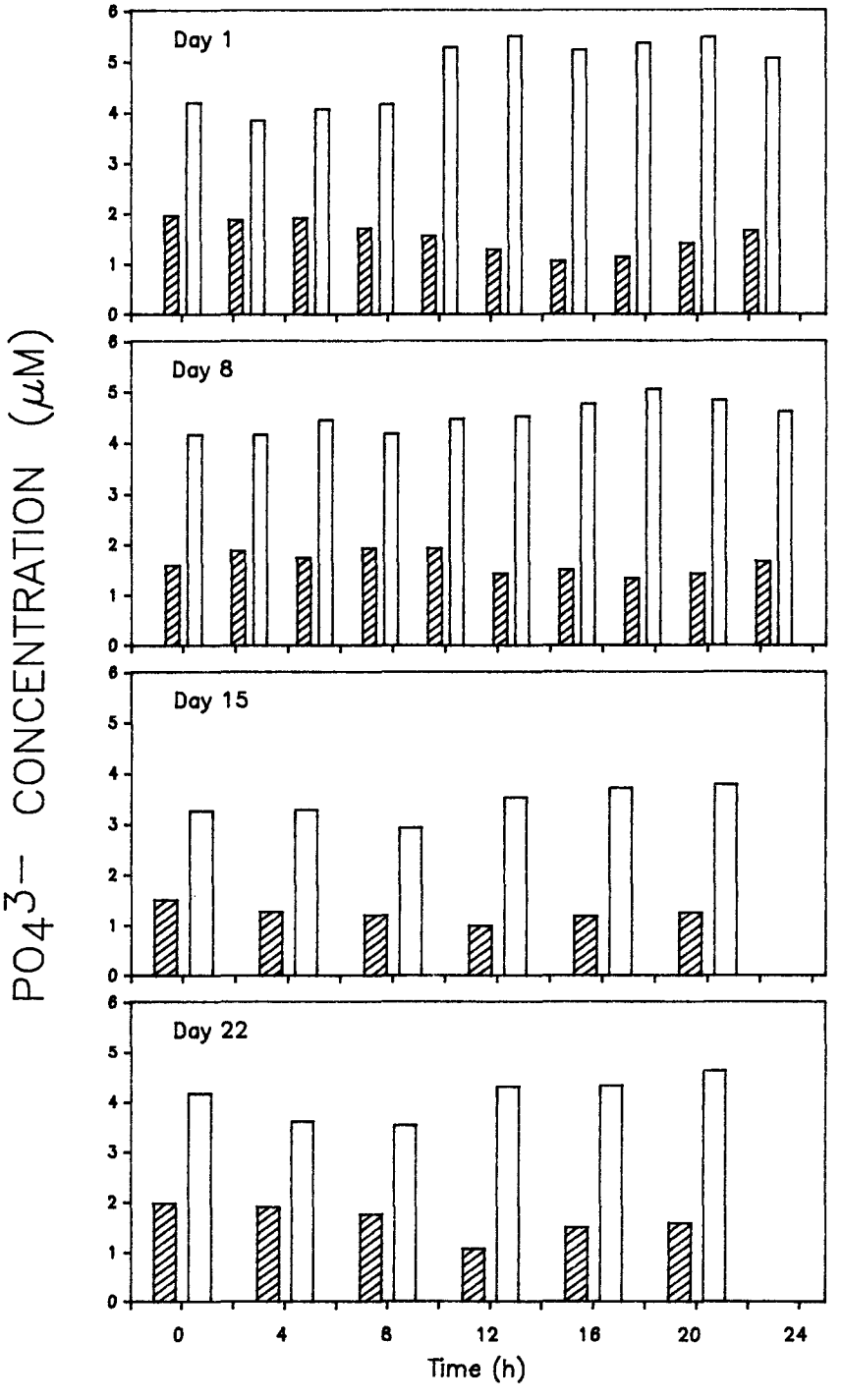


Figure 5. $[PO_4^{3-}]$ of stripped seawater (▨) and salmon effluent (□) during limiting factor experiment. Sampling period: 19 October – 9 November 1989. Fish fed at 8:00.

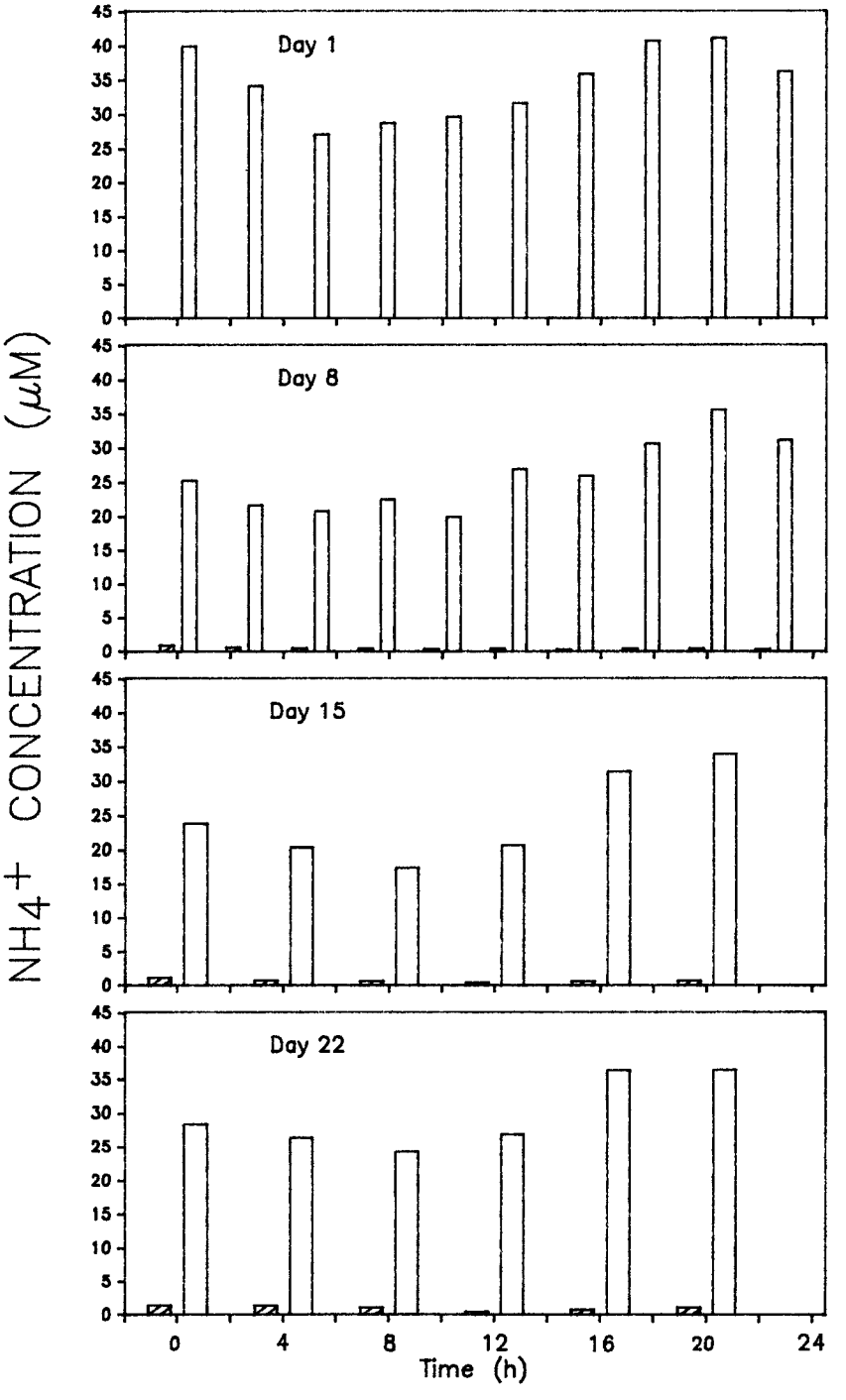


Figure 6. [$NH_4^+$] of stripped seawater (▨) and salmon effluent (▭) during limiting factor experiment. Sampling period: 19 October – 9 November 1989. Fish fed at 8:00.

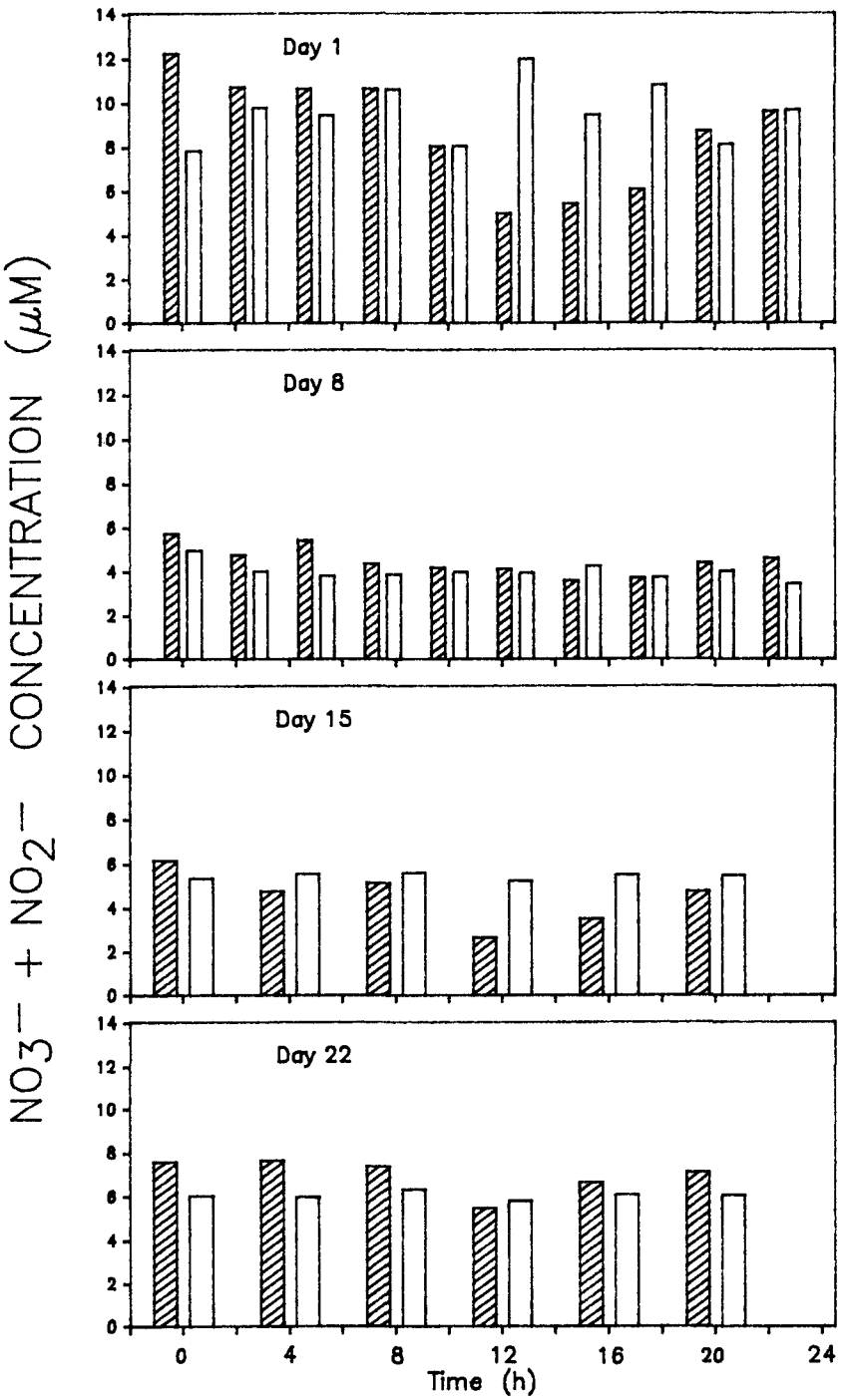


Figure 7. $[NO_3^- + NO_2^-]$ of stripped seawater (▨) and salmon effluent (▭) during limiting factor experiment. Sampling period: 19 October – 9 November 1989. Fish fed at 8:00.

one another. C, N, and P accumulation of P. mollis during the Limiting Factor experiment are presented in Figure 8; nutrient ratios are presented in Figure 9.

A highly significant inverse relationship was found between irradiance level and tissue C (ANOVA, $F = 15.89$, $df = 3$, $p < 0.0001$) and N (ANOVA, $F = 9.00$, $df = 3$, $p < 0.001$) concentrations (Fig. 8). This suggests that at higher light levels plants were not storing nutrients but utilizing them for production of biomass. No such trend was found for P (ANOVA, $F = 0.90$, $df = 3$), suggesting that need for this nutrient for growth was not as great as for N and C. Sufficient amounts of P necessary for good growth existed in plant tissue (Fig. 8). Increased nutrient loading did not lead to higher tissue N levels (ANOVA, $F = 1.97$, $df = 1$). An increase in nutrient loading did, however, result in higher C (ANOVA, $F = 5.81$, $df = 1$, $p < 0.05$) and P (ANOVA, $F = 5.41$, $df = 1$, $p < 0.05$) levels in P. mollis tissue within a light treatment. No significant difference was detected between nutrient loading for C (ANOVA, $F = 2.17$, $df = 3$) or P (ANOVA, $F = 0.10$, $df = 3$) levels between light treatments.

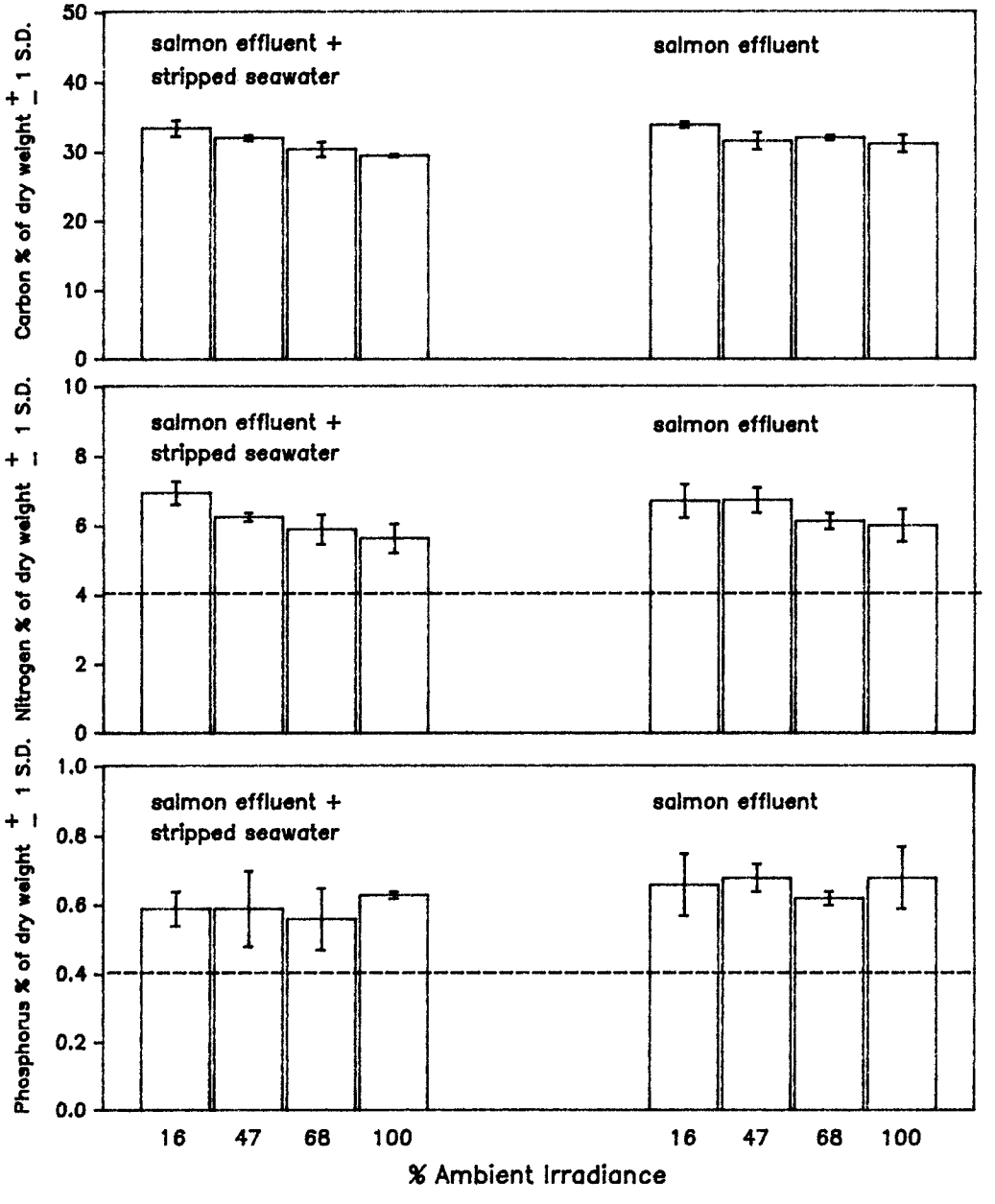


Figure 8. Effect of nutrient regime and irradiance level on accumulation of C, N, and P by P. mollis during limiting factor experiment. Tissue samples collected on Day 15 of experiment. Values above dashed line are reported in literature to be in excess of concentrations necessary for non-nutrient limited growth.

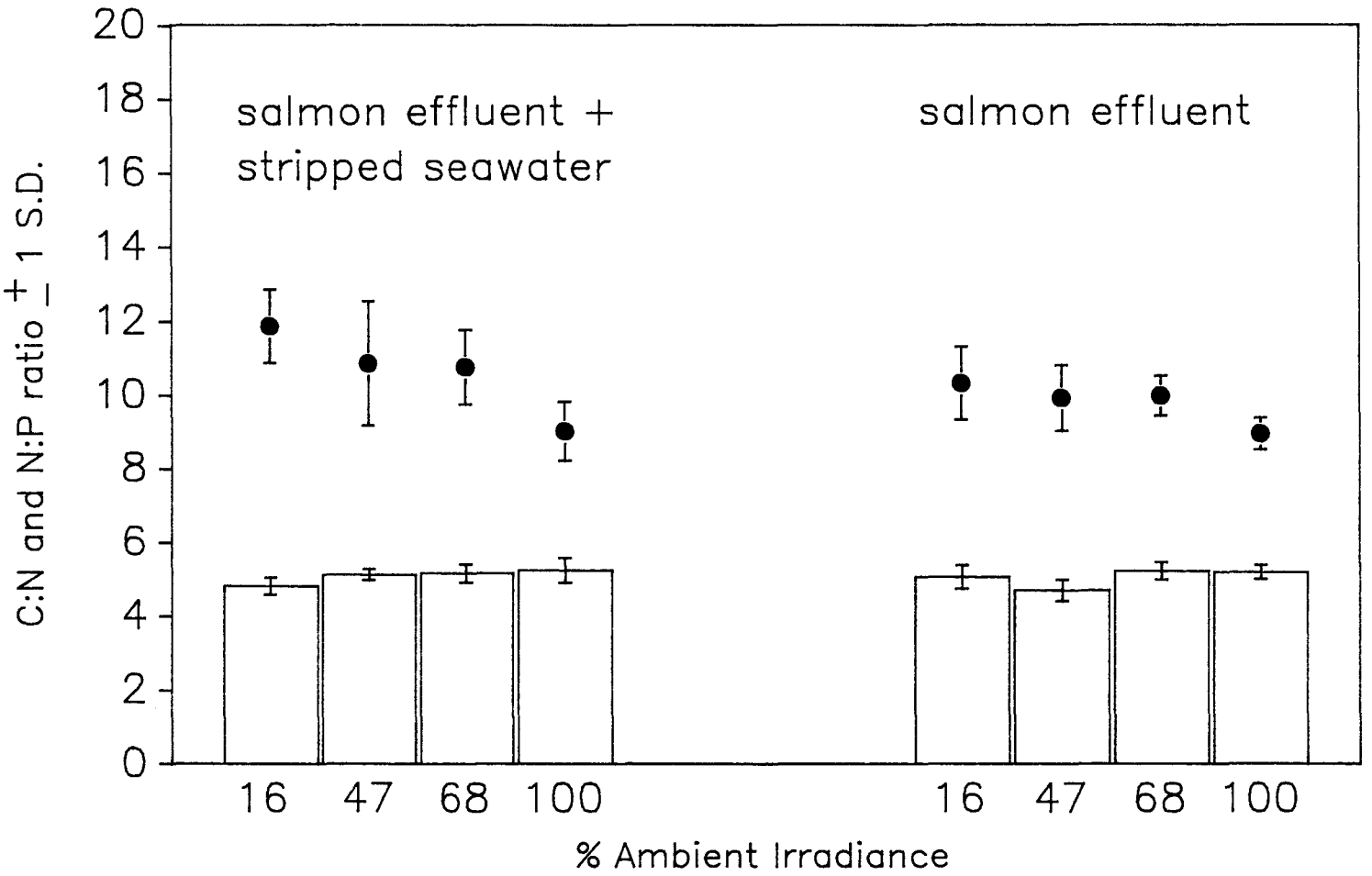


Figure 9. Effect of nutrient regime and irradiance level on C:N (▭) and N:P (●) ratios for P. mollis tissue collected on Day 15 of limiting factor experiment.

## DISCUSSION

Growth rates of P. mollis observed in this study were comparable to those of P. palmata reported by others. During the Stocking Density experiment of this study, an average SGR of 5.3 was observed for apical and basal portions of P. mollis stocked at 5.65 g $l^{-1}$ and exposed to approximately 2.8 x $10^6$ J $m^{-2}$ $d^{-1}$ of natural daylight. Morgan and Simpson (1981a) reported an SGR of 6 for fast growing apical segments of P. palmata at a stocking density of 6.25 g $l^{-1}$ and a natural daylight irradiance level of approximately 2.1 x $10^6$ J $m^{-2}$ $d^{-1}$. In an examination of the growth response of P. palmata to various concentrations and sources of inorganic N, an average SGR of 4.5 was obtained using apical segments stocked at 6.25 g $l^{-1}$ and receiving 3.6 x $10^6$ J $m^{-2}$ $d^{-1}$ from cool-white fluorescent lamps (Morgan and Simpson, 1981b).

The optimum stocking density for P. mollis will probably vary seasonally. Waaland (1978) made monthly adjustments of the stocking densities of I. cordata and G. exasperata based on ambient photoperiod and irradiance levels at the culture site in Seattle, Washington. Monthly stocking density was expressed as a percentage of the stocking density experimentally determined to be optimal for June and July, the months of peak solar radiation. For example, the average solar radiation for April was 70% of that for June and July; therefore the stocking density recommended for April was 70% of that for June and July.

Since the Stocking Density experiment of this study was conducted in April/May, the optimum stocking density should be higher in months of greater solar radiation.

The concept of altering the stocking density to account for changes in solar radiation is supported by results of the Stocking Density and Limiting Factor experiments of this study. This is best illustrated by comparing the SGR of P. mollis at a stocking density of 10.17 g $l^{-1}$ from the Stocking Density experiment (early March - early May) to that of algae stocked at the same density and with the same light screening (16% of ambient) from the Limiting Factor experiment (early October - early November). SGR of P. mollis during the Stocking Density experiment was about three times greater than during the Limiting Factor experiment (SGR = 3.49 vs SGR = 1.12). Correspondingly, the ambient irradiance level during April/May was approximately five times greater than that during October/November. Observed growth response differences were likely to be a factor of ambient irradiance levels. Mean daily solar radiation during each month of the year measured at Coos Bay, Oregon (located approximately 88 miles south of the Hatfield Marine Science Center) is presented in Figure 10. Monthly values are expressed as a percentage of the July value, as July was the month of peak solar radiation. Stocking densities of algae cultured at the Hatfield Marine Science Center could

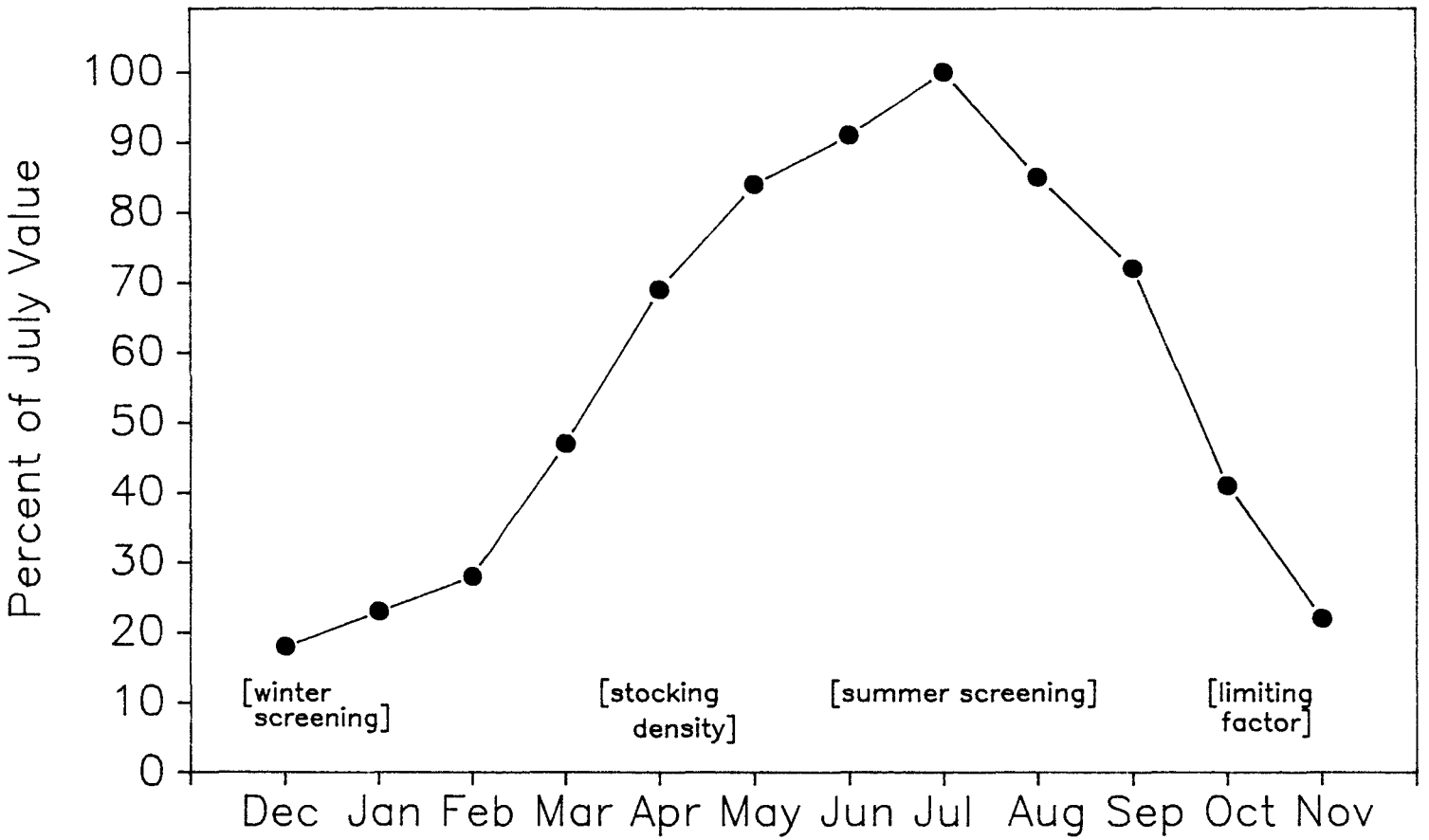


Figure 10. Average daily solar radiation for each month of the year measured at Coos Bay, Oregon, 1980 – 1988. Values standardized to July mean of $23.6 \times 10^{6}$ J $m^{-2}$ $d^{-1}$.

be adjusted monthly by these percentages as was proposed by Waaland (1978).

The Limiting Factor experiment confirmed that in a nutrient regime which was typical of the effluent of a land-based salmon facility, the SGR of P. mollis was a function of ambient solar radiation (Fig. 4). Morgan and Simpson (1981c) observed that P. palmata grew fastest at higher light levels when cultured at identical stocking densities and nutrient regimes but varying irradiance levels ($8.0 \times 10^5$, $2.2 \times 10^6$, and $3.6 \times 10^6$ J $m^{-2}$ $d^{-1}$). Maximum productivity during the Limiting Factor experiment was 15.7 g dry weight $m^{-2}$ $d^{-1}$. Productivity approaching 50 g dry weight $m^{-2}$ $d^{-1}$ may be realized during the month of peak solar radiation (July). This is based on an SGR of 8, algal stocking density of 14.75 g $l^{-1}$, and an average dry weight to wet weight ratio of 0.1425 for P. mollis during this study. The 14.75 g $l^{-1}$ stocking density is derived by multiplying the stocking density determined to be optimum in April (10.17 g $l^{-1}$) by the ratio of July:April irradiance levels (1.45). Morgan and Simpson (1981c) obtained dry weight to wet weight ratios for P. palmata as high as 0.2720 by increasing irradiance levels and decreasing N enrichment. Therefore, productivity of cultured P. mollis could be increased an additional 91% over the 50 g dry weight $m^{-2}$ $d^{-1}$ value derived above by managing irradiance and nutrient regimes. Productivity of

P. mollis in this study compares favorably to that of other marine macroalgae and terrestrial crops (Table 6).

Tissue nutrient levels obtained from the Limiting Factor experiment are the most reliable indicator that growth of macroalgae was light limited and not nutrient limited. Low N:P ratios evident in Figure 9 indicate an abundance of P, not N limitation. C:N ratios less than 10 presented in Figure 9 are an indication of surplus N (D'Elia and DeBoer, 1978); P. mollis C:N ranged from 4.7 to 5.25 in the Limiting Factor experiment of this study. Figure 8 reveals that both N and P concentrations in P. mollis tissue collected during the Limiting Factor experiment are beyond that required for growth. Tissue N levels greater than 4% of algal dry weight and tissue P levels greater than 0.4% of algal dry weight are considered to be surplus amounts (Bjornsater and Wheeler, in press). N and P tissue levels from the Limiting Factor experiment ranged from 5.3 - 7.3% and 0.47 - 0.77% algal dry weight, respectively. An inverse relationship existed between tissue N level and light level. Accumulation of N by macroalgae when their growth is limited by low light levels has been noted in both Rhodophyta (Morgan and Simpson, 1981a,c) and Chlorophyta (Lapointe and Tenore, 1981). This study indicated that growth of P. mollis cultured in salmon effluent was limited by light, not by nutrient availability.

Table 6. Productivity of various marine macroalgae and agricultural crops.

| Macroalgal Species | Culture Conditions | Stocking Density (kg wet wt $m^{-2}$) | Productivity (g dry wt $m^{-2}$ $d^{-1}$) |
|---|---|---|---|
| *Chondrus crispus*[1] | tank culture<br>N. French coast | | |
| | Aug | 5.1 | 15.4 |
| | Apr | 4.0 | 7.5 |
| | Jan | 2.0 | 1.4 |
| *C. crispus*[2] | Nova Scotia, Canada | 4-6 | 25-35 |
| *Eucheuma striatum*[3] | half-hectare ponds<br>Philippines | | |
| | Dec-Jul | 12-14 | - |
| *Gelidium coulteri*[4] | tank culture<br>Hopkins Marine Station, CA | 17 | 2.7 |
| *G. coulteri*[4] | tank culture<br>Marine Bioassay Laboratories | 10-16 | 3.6 |
| *Gigartina exasperata*[5] | semi-closed culture | | |
| | 1974 - wild type | 5.2 | - |
| | 1976 - M-11 strain | 9.0 | - |
| *Gracilaria* sp.[6] | natural algal beds<br>Vancouver Is., B.C., Canada | | |
| | sheltered intertidal | 4.7 | 4.8 |
| | exposed subtidal | 2.0 | 0.1 |
| *Gracilaria* sp.[7] strain G-16 | 880 l tanks<br>Ft. Pierce, FL | | |
| | May-Jul | 21 | 2.0 |

(continued)

Table 6 - - continued

| Macroalgal Species | Culture Conditions | Stocking Density (kg wet wt $m^{-2}$) | Productivity (g dry wt $m^{-2}$ $d^{-1}$) |
|---|---|---|---|
| G. foliifera[8] | tank culture | | |
| | Jan-Feb | 0.8 | 24 |
| | Mar-Apr | 0.6 | 7 |
| G. secundata[9] | 2000 l cylinder w/ constant submerged light. algae uncropped over 13 d trial | 1 g $l^{-1}$ initial | 76 |
| G. sjoestedtii[4] | tank culture Elkhorn Slough, CA | 3.5 | 12-14 |
| G. tikvahiae[10] | 600 l tanks Ft. Pierce, FL | | |
| | Aug | 1.5 | 34.5 |
| G. verrucosa[11] | pond culture Taiwan | 0.6 | 4-12 |
| Iridaea cordata[12] | natural algal beds central CA coast | | |
| | winter | - | 0.3 |
| | spring | - | 7 |
| | summer | - | 19 |
| I. cordata[13] | net culture San Juan Is., WA various harvest regimes | | |
| | Mar-Jul | - | 14-21 |
| I. cordata[5] | natural algal beds | | |
| | Pacific Northwest | - | 1.7 |
| | California | - | 6.3 |

(continued)

Table 6 - - continued

| Macroalgal Species | Culture Conditions | Stocking Density (kg wet wt $m^{-2}$) | Productivity (g dry wt $m^{-2}$ $d^{-1}$) |
|---|---|---|---|
| *I. cordata*[5] | semi-closed culture | | |
| | 1974 | - | 10.8 |
| | 1976 | - | 11.9 |
| *Neoagardhiella baileyi*[8] | tank culture | | |
| | Mar-Apr | 0.6 | 7 |
| *Palmaria palmata*[14] | 8 l tank culture fluorescent light summer photoperiod | 1.1 | 18 |
| *P. palmata*[15] | 255,000 l ponds Grand Manan Is., New Brunswick | 10 | 60 |
| *P. mollis*[16] | 11.5 l tank culture Newport, OR Oct-Nov | 0.9 | 15 |
| *Ulva lactuca*[17] | 700 l tanks Ft. Pierce, FL | | |
| | aerated | 1.0 | 19 |
| | unaerated | 1.0 | 7 |
| Wheat[5] | Netherlands | - | 7.4 |
| Corn[5] | United States | - | 7.1 |
| Rice[5] | Japan | - | 8.5 |
| Sugar Cane[5] | Hawaii | - | 18.3 |

Reference:

1) Braud and Delepine, 1981
2) Hansen et al., 1981
3) Doty and Alvarez, 1981
4) Hansen, 1983
5) Waaland, 1978
6) Saunders and Lindsay, 1979
7) Guerin and Bird, 1987
8) DeBoer, 1979
9) Lignell et al., 1987
10) DeBusk and Ryther, 1984
11) Chiang, 1981
12) Hansen, 1981
13) Mumford, 1978
14) Morgan and Simpson, 1981a
15) Neish, 1976
16) This study
17) DeBusk et al., 1986

Cost effectiveness of supplemental lighting on a commercial scale to enhance macroalgal growth is likely to be site specific, depending upon local power rates, increment in macroalgal yield per unit energy cost, and crop value. Lignell et al. (1987) demonstrated that for Gracilaria secundata a SGR of 47 could be obtained by submerging lights in the culture vessel. Growth rates in their system were positively correlated with flux densities up to 1450 $\mu E\ m^{-2}\ s^{-1}$. This response is more likely to occur with intertidal species than subtidal species such as P. mollis (Lobban et al., 1985). Robbins (1979) observed photosynthesis of P. palmata to be saturated at 212 $\mu E\ m^{-2}\ s^{-1}$. Morgan and Simpson (1981a) determined that if P. palmata is cultured so that self-shading is minimal (using large, shallow tanks or bubble culture), approximately 245 $\mu E\ m^{-2}\ s^{-1}$ of light per kg fresh weight of algae per $m^2$ is required to obtain a maximum SGR of 8.

The predominance of $NH_4^+$ as the N source in salmon effluent is beneficial to macroalgal growth (Lobban et al., 1985). DeBoer et al. (1978) found $NH_4^+$ to be superior to $NO_3^-$ as a N source for Gracilaria foliifera and Neoagardhiella baileyi growth (max. $[NH_4^+]$ = 38 $\mu M$). In contrast, Morgan and Simpson (1981b) found $NO_3^-$ to stimulate higher growth of P. palmata than $NH_4^+$ even though $NH_4^+$ uptake and accumulation was greater than that for $NO_3^-$. However, the high concentrations of $NH_4^+$ used by these authors (0.5 - 2.0 mM) may have been toxic to the

plants. D'Elia and DeBoer (1978) reported that $NO_3^-$ uptake by G. foliifera and N. baileyi was reduced at $[NH_4^+]$ as low as 5 µM.

Epiphytes are expected to be the greatest problem which must be overcome before P. mollis can be produced commercially for human consumption in land-based salmon mariculture facilities. Although P. mollis was more resistant than other species of macroalgae during this study, diatoms did grow on basal portions of plants. Epiphytism by diatoms is likely to have a greater impact on the market value of P. mollis than species cultured for their hydrocolloid products. P. mollis is principally marketed as a dried product (dulse) for human consumption. When dried, diatoms attached to dulse turn an olive-green color. Since color is the attribute which determines grade and therefore value of dulse, plants epiphytized by diatoms will have a lower market value than plants which are not epiphytized.

As epiphytes are the greatest obstacle to commercial production of seaweeds (Nelson et al., 1980), their control has been the subject of much research. Among the methods used to reduce epiphytes are pulse feeding (Morgan and Simpson, 1981b; DeBusk and Ryther, 1984), extended exposure to air (emergence) (Davis, 1980b; Mumford and Waaland, 1980; Hansen, 1984), sterilization with dilute chemical solutions (Mumford and Waaland, 1980; G. Hansen, pers.

comm.), fresh or saltwater rinsing (Mumford and Waaland, 1980), decreasing light intensity, which may be accomplished by shading with screens or maintaining high crop stocking densities (Enright, 1979; Morgan and Simpson, 1981a), low culture temperatures (Enright, 1979; Morgan and Simpson, 1981a), and grazers such as snails (Mumford and Waaland, 1980), fish, penaid shrimp (Nelson et al., 1980), isopods, and amphipods (Shacklock and Doyle, 1983). Of the two reported techniques used for control of epiphytes on P. palmata, emergence (Davis 1980b) and isopods/amphipods (Shacklock and Doyle, 1983), emergence may be the only suitable option as the crustaceans also grazed extensively on Palmaria.

Given that the epiphyte problem can be resolved or minimized, P. mollis is a prime candidate for integration into a land-based polyculture system. This species has demonstrated rapid, sustained growth during experiments conducted over most of the year. Under natural conditions, P. palmata can only be harvested manually when it is accessible in its rocky, subtidal habitat. Its availability in the market is therefore limited. A cultured crop of consistent quality could help meet market demand.

Ease of culture also favors selection of P. mollis as a cultured species. P. mollis used in this study continued to grow vegetatively with no evidence of senescence or

sexual reproduction; therefore, no control of sexual reproduction seems to be necessary. Appearance of this species can also be used as an indicator of culture conditions. Pale red to pink thalli indicate a shortage of nitrogen, while a yellow of green color indicates that the plant is not receiving enough light. On a commercial scale, periodic tank maintenance, harvesting excess material to return stocking densities to optimal levels, and processing harvested material for distribution are the only labor requirements.

Preparing dulse for market is a very simple process. Harvested surplus is simply spread out to dry and then packaged for bulk distribution. A greenhouse or other drying building may be necessary to ensure proper drying during periods of rain. The dried product falls into one of two grades. Plants that are an even burgundy color and have no precipitate on their surface (an indicator of a prolonged drying period) are categorized as grade I. All other material is categorized as grade II. Grade I plants are marketed in a whole leaf form, while grade II dulse is distributed as flakes or powder. Current prices paid by Maine Coast Sea Vegetables (Franklin, ME) and Granum (Seattle, WA), the principal dulse buyers, are \$4.50 $lb^{-1}$ dry weight for grade I and \$3.50 $lb^{-1}$ dry weight for grade II. Cultured dulse could bring a higher price to producers who become involved in packaging and distributing their own

product. Wholesale prices of dulse are currently \$12.12 $lb^{-1}$ dry weight for whole leaf or powder, and \$11.66 $lb^{-1}$ dry weight for flakes. The same price is paid for the powdered form as the whole leaf product due to increased processing costs. However, retail demand is predominantly for the whole leaf form.

While this study has shown P. mollis was the species best suited for culture in land-based salmon effluent in temperate waters, the concept of macroalgae/fish polyculture could be applied to tropical pump-ashore facilities where inorganic nitrogenous wastes are produced by crops of fish or shellfish. Sales of macroalgae could help offset operating costs of land-based aquaculture facilities, thereby increasing their economic viability. Since the costs of pumping water and feeding the principal crop (fish or shellfish) are an intrinsic part of the polyculture system, added costs of producing macroalgae are likely to represent a relatively minor portion of total costs. Conservative projections of the annual yield of P. mollis cultured on a commercial scale in Oregon reveal that its net value per unit surface area is greater than that of the salmon component of the polyculture system (W. McNeil, pers. comm.).

Effluent from fish or shellfish ponds passed through seaweed cultures will be lower in dissolved inorganic nutrients and higher in dissolved oxygen than untreated

effluent, thereby decreasing the risk of eutrophication of receiving waters. This is an added benefit resulting from the integration of macroalgae into pump-ashore mariculture systems, especially at locations where nutrient addition to natural waters is considered to be a pollution problem by regulatory agencies or the public.

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# APPENDIX

## Nutrient analyses reagent recipes

$PO_4^{3-}$

Ammonium molybdate solution - Dissolve 15 g ammonium paramolybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (analytical reagent) in 500 ml deionized water. Store in a plastic bottle out of direct sunlight. Solution is stable indefinitely.

Sulfuric acid solution - Add 140 ml concentrated sulfuric acid (analytical reagent, sp. gr. 1.82) to 900 ml deionized water. Store in a glass bottle.

Ascorbic acid solution - Dissolve 27 g ascorbic acid in 500 ml deionized water. Store in a plastic bottle and keep frozen between uses.

Potassium antimonyl tartrate (tartar emetic) - Dissolve 0.34 g in 250 ml deionized water, warming if necessary. Solution is stable for many months.

Mixed reagent - Mix together 2 parts ammonium molybdate, 5 parts sulfuric acid, 2 parts ascorbic acid, and 1 part potassium antimonyl tartrate solutions. Prepare for immediate use only, and discard excess. Do not store for more than six hours.

$NH_4^+$

High-quality deionized water - obtained from Milli-Q ion exchange system.

Phenol solution - Dissolve 20 g of crystalline analytical-reagent-grade phenol in 200 ml of 95% (v/v) ethyl alcohol.

Sodium nitroprusside solution - Disodium nitroprusside dihydrate $Na_2Fe(CN)_5NO \cdot 2H_2O$ (sodium ferricyanide), should be recrystallized to remove impurities and to produce finer crystals that facilitate weighing small amounts. This is achieved by dissolving about 5 g in 20 ml deionized water, adding 400 ml 95% ethanol, storing at -5 to $0^{\circ}C$ overnight, collecting crystals on filter paper, followed by complete drying. The reagent solution is made by dissolving 1.0 g of recrystallized sodium nitroprusside in 200 ml of deionized water. This solution should be stored in a refrigerator in a brown glass bottle and is stable for at least one month.

Alkaline reagent - Dissolve 100 g of sodium citrate and 5 g of sodium hydroxide (analytical reagent) in 500 ml of deionized water. This solution is stable indefinitely.

Sodium hypochlorite solution - Use a solution of commercial hypochlorite (e.g., Chlorox), which should be about 1.5 N. Strickland and Parsons (1972) describe procedures for checking solution strength.

Oxidizing solution - Mix 4 parts alkaline reagent and 1 part hypochlorite solution. Keep stoppered when not in use and prepare fresh daily.

$NO_3^- + NO_2^-$

Sulfanilamide solution - Dissolve 5 g sulfanilamide in 50 ml concentrated HCl and 300 ml deionized water; dilute to final volume of 500 ml. Solution is stable for months.

N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDA) solution - Dissolve 0.5 g in 500 ml of distilled water and store in brown bottle in the refrigerator. Replace stock solution monthly or whenever a brown color develops.